FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 86450

U.S APPLICATION NO (IF KNOWN, SEE 37 CFR): 10/070181

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/01011	30/08/2000	30/08/1999

TITLE OF INVENTION
A NUTRITIONAL SUPPLEMENT FOR LOWERING SERUM TRIGLYCERIDE AND CHOLESTEROL LEVELS

APPLICANT(S) FOR DO/EO/US
JEFFREY L.C. WRIGHT AND JAROSLAV A. KRALOVEC

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

Rec'd PCT/PTO 27 FEB 2002

APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070181	PCT/CA00/01011	86450

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	22 - 20 =	2	x $18.00	$36.00
Independent claims	2 - 3 =	0	x $84.00	$0.00
Multiple Dependent Claims (check if applicable).			☐	$0.00
TOTAL OF ABOVE CALCULATIONS =				$926.00
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$0.00
SUBTOTAL =				$926.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$0.00
TOTAL NATIONAL FEE =				$926.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☒				$40.00
TOTAL FEES ENCLOSED =				$966.00
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$966.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **23-0920** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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312-655-1500
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Gerald T. Shekleton
SIGNATURE

GERALD T. SHEKLETON
NAME

27, 466
REGISTRATION NUMBER

2/27/02
DATE

Commissioner for Patents
Washington, D.C. 20231

Preliminary Amendment

Sir:

By way of a preliminary amendment on the above-noted application for patent, please consider the following amendments and remarks.

Amendment

IN THE DESCRIPTION:

Please add the following paragraph to page 1 just after the title and before the Field of the Invention:

"Related Applications

This application is a National Entry of International Application No. PCT/CA00/01011 filed on August 30, 2000 and claims the benefit of United States Application USSN 09/385,834 filed on August 30, 1999."

IN THE CLAIMS:

Please cancel claims 1-45 without prejudice to the Applicant's right to file one or more divisional applications.

Please add new claims 46-67 as follows:

46. (New) A process for preparing an ester between a sterol and eicosapentaenoic acid 20:5ω3 (EPA), docosahexaenoic acid 22:6ω3 (DHA) or a mixture of EPA and DHA, which comprises the step of reacting the sterol with esters of EPA, DHA or a mixture of EPA and DHA in the presence of a base.

47. (New) The process according to claim 46, wherein the base is a metal (C_1-C_{10})alkoxide.

48. (New) A process according to claim 47, wherein the metal (C_1-C_{10})alkoxide is sodium methoxide.

49. (New) The process according to claim 47, which further comprises the step of precipitating unreacted sterol with a suitable solvent, and filtering off the precipitated unreacted sterol to leave a filtrate.

50. (New) The process according to claim 49, wherein the solvent is hexane.

51. (New) The process according to claim 50, which further comprises the step of extracting the filtrate with a suitable immiscible solvent to remove unreacted esters of EPA, DHA or mixture thereof, from the filtrate.

52. (New) A process according to claim 51, wherein the immiscible solvent is methanol.

53. (New) A process according to claim 52, wherein the ester of EPA, DHA or mixture thereof is a triglyceride ester.

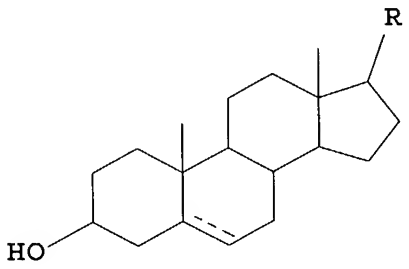
54. (New) A process according to claim 53, wherein the ester of EPA, DHA or mixture thereof is an ethyl ester.

55. (New) A process according to claim 52, wherein the EPA, DHA or mixture thereof is derived from fish oil.

56. (New) A method comprising: administering a blood serum cholesterol and triglyceride lowering effective amount of an ester formed between a sterol and eicosapentaenoic acid 20:5ω3 (EPA), docosahexaenoic acid 22:6ω3 (DHA) or a mixture of EPA and DHA, to a subject in need thereof.

57. (New) The method according to claim 56, wherein the sterol is a phytosterol.

58. (New) The method according to claim 56, wherein the sterol has the formula (I):



(I)

wherein R is a (C_1-C_{10})alkyl, substituted (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl or substituted (C_2-C_{10})alkenyl group and the dashed line indicates that a double or single bond may exist at that location in the sterol.

59. (New) The method according to claim 56, wherein the sterol is stigmasterol.

60. (New) The method according to claim 56, wherein the sterol is sitosterol.

61. (New) The method according to claim 56, wherein the sterol is fucosterol.

62. (New) The method according to claim 56, wherein the sterol is fucostanol.

63. (New) The method according to claim 56, wherein the sterol is β-sitostanol.

64. (New) The method according claim 56, wherein a mixture of EPA and DHA forms the ester with the sterol.

65. (New) The method according to claim 56, wherein the EPA, DHA or mixture thereof is derived from fish oil.

66. (New) The method according to claim 56, wherein the ester is administered in the form of a nutritional supplement.

67. (New) The method according to claim 66, wherein the nutritional supplement further comprises an edible additive.

Remarks

The Applicant has added related application data to page 1 of the specification.

Claims 1-45 were pending before this communication. By this amendment, the Applicant has also provided a new set of claims which more particularly set forth the invention. New claims 46-55 are directed to processes for preparing an ester between a sterol and EPA, DHA or a mixture of EPA and DHA. Claims 56-67 are directed to methods for lowering cholesterol and triglyceride levels in the blood of a subject in need of such lowering by administering an ester between a sterol and EPA, DHA or a mixture of EPA and DHA. The new claims add no subject matter as they are fully supported by the specification and the original claims. A clean copy of the complete set of pending claims is attached as Appendix A.

Conclusion

In view of the above amendment and remarks, consideration of the new set of claims is respectfully requested.

Respectfully submitted,

Date: 2/15/02

Gerald T. Shekleton

Enclosure: Appendix A

Appendix A – Clean Copy of Pending Claims

46. (New) A process for preparing an ester between a sterol and eicosapentaenoic acid 20:5ω3 (EPA), docosahexaenoic acid 22:6ω3 (DHA) or a mixture of EPA and DHA, which comprises the step of reacting the sterol with esters of EPA, DHA or a mixture of EPA and DHA in the presence of a base.

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48. (New) A process according to claim 47, wherein the metal (C_1-C_{10})alkoxide is sodium methoxide.

49. (New) The process according to claim 47, which further comprises the step of precipitating unreacted sterol with a suitable solvent, and filtering off the precipitated unreacted sterol to leave a filtrate.

50. (New) The process according to claim 49, wherein the solvent is hexane.

51. (New) The process according to claim 50, which further comprises the step of extracting the filtrate with a suitable immiscible solvent to remove unreacted esters of EPA, DHA or mixture thereof, from the filtrate.

52. (New) A process according to claim 51, wherein the immiscible solvent is methanol.

53. (New) A process according to claim 52, wherein the ester of EPA, DHA or mixture thereof is a triglyceride ester.

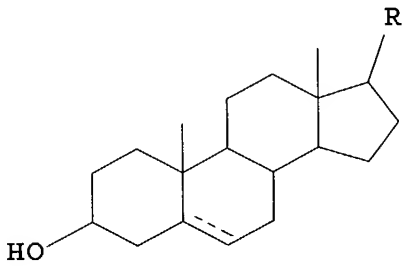
54. (New) A process according to claim 52, wherein the ester of EPA, DHA or mixture thereof is an ethyl ester.

55. (New) A process according to claim 52, wherein the EPA, DHA or mixture thereof is derived from fish oil.

56. (New) A method comprising: administering a blood serum cholesterol and triglyceride lowering effective amount of an ester formed between a sterol and eicosapentaenoic acid 20:5ω3 (EPA), docosahexaenoic acid 22:6ω3 (DHA) or a mixture of EPA and DHA, to a subject in need thereof.

57. (New) The method according to claim 56, wherein the sterol is a phytosterol.

58. (New) The method according to claim 56, wherein the sterol has the formula (I):



(I)

wherein R is a (C_1-C_{10})alkyl, substituted (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl or substituted (C_2-C_{10})alkenyl group and the dashed line indicates that a double or single bond may exist at that location in the sterol.

59. (New) The method according to claim 56, wherein the sterol is stigmasterol.

60. (New) The method according to claim 56, wherein the sterol is sitosterol.

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62. (New) The method according to claim 56, wherein the sterol is fucostanol.

63. (New) The method according to claim 56, wherein the sterol is β-sitostanol.

64. (New) The method according claim 56, wherein a mixture of EPA and DHA forms the ester with the sterol.

65. (New) The method according to claim 56, wherein the EPA, DHA or mixture thereof is derived from fish oil.

66. (New) The method according to claim 56, wherein the ester is administered in the form of a nutritional supplement.

67. (New) The method according to claim 66, wherein the nutritional supplement further comprises an edible additive.

A Nutritional Supplement For Lowering Serum Triglyceride and Cholesterol Levels

Field of the Invention

The invention relates to control of cholesterol and triglyceride levels in mammals, particularly humans.

Background of the Invention

Serum cholesterol and serum triglyceride levels are important factors in the development of cardiovascular disease. In many clinical studies there is a positive correlation between plasma triglycerides and the incidence of cardiovascular disease [1]. Elevated plasma triglyceride level is frequently associated with other atherogenic factors including elevated low-density lipoprotein (LDL)-cholesterol, reduced high-density lipoprotein (HDL)-cholesterol, and small LDL particles [2, 3]. There is growing acceptance that triglycerides act in a synergistic fashion with these other lipid risk factors to increase the incidence of cardiovascular disease [4, 5]. Hypertriglyceridemia usually occurs because of insulin resistance, which leads to overproduction of very low-density lipoproteins (VLDL) by the liver [3]. Treatment involves lifestyle changes to decrease body weight and to increase physical activity, both of which improve insulin sensitivity. Drug therapy to lower triglycerides involves the use of fibrates or nicotinic acid [6].

A number of clinical studies convincingly establish plasma cholesterol and LDL-cholesterol as independent risk factors for coronary heart disease [7]. Pharmacological agents, called statins, lower total plasma cholesterol by inhibiting the synthesis of cholesterol by the liver. The

statins reduce the morbidity and mortality rate from cardiovascular disease in high risk, hypercholesterolemic patients [8, 9], but also in persons who exhibit "average" cholesterol levels [10]. Another approach is to interfere with the intestinal absorption of cholesterol. Certain phytosterols (plant sterols) such as stigmasterol and β-sitosterol lower serum cholesterol act by inhibiting absorption of both dietary and biliary cholesterol from the small intestine [11].

With respect to the most appropriate form of phytosterols for lowering serum cholesterol, some reports indicate that free phytosterols reduce serum cholesterol in animals and humans [12, 13]. However, there is also evidence to indicate that a sterol esterified with a fatty acid may be more effective [14]. Trials show that phytosterol esters of plant fatty acids obtained from canola oil, when incorporated into food such as margarine or mayonnaise, lower total cholesterol and LDL-cholesterol levels by about 10 and 15 percent, respectively [15, 16]. United States Patent No. 5,502,045 (Miettinen et al., issued March 26, 1996) discloses the use of sitostanol esters of canola oil to lower serum cholesterol. Benecol™ (Raisio Benecol Ltd., Raisio, Finland), a margarine that contains such compounds, is now on the market.

The mechanism by which phytosterols or phytosterol esters inhibit absorption of dietary cholesterol by the digestive tract is not fully understood but may involve competitive inhibition of cholesterol uptake from the intestinal lumen or inhibition of cholesterol esterification in the intestinal mucosa [12]. It is known that phytosterols themselves are only poorly absorbed. Vanhanen et al. [17] report that phytosterol esters may also be poorly absorbed by

the intestinal tract based on postprandial measurements of β-sitostanol in plasma. A direct measure of phytosterol ester uptake by the digestive tract has not been reported.

When phytosterols are esterified with fatty acids from plant sources such as canola, the long-chain polyunsaturated fatty acids (LCPUFAs) that are incorporated are predominantly of the omega-6 series. Omega-6 fatty acids do not affect plasma triglycerides. Research to date on fatty acid esters of sterols has focused only on the efficacy of the sterol in lowering cholesterol.

Summary of the Invention

The present invention provides a nutritional supplement comprising a sterol and an omega-3 fatty acid, or an ester thereof, for lowering cholesterol and triglyceride levels in the bloodstream of a subject.

The present invention also provides a method of lowering cholesterol and triglyceride levels in the bloodstream of a subject, the method including the step of administration of an effective amount of a nutritional supplement comprising a sterol and an omega-3 fatty acid, or an ester thereof, to a subject.

The present invention also provides the use of the nutritional supplement defined herein for lowering cholesterol and triglyceride levels in the bloodstream of a subject.

The subject is preferably a mammal, more preferably a human.

The present invention further provides a foodstuff composition comprising the nutritional supplement defined

herein and a foodstuff, the nutritional value of the foodstuff being enhanced by incorporation of the nutritional supplement defined herein.

The present invention further provides the use of the nutritional supplement defined herein in the manufacture of a foodstuff composition.

The present invention further provides a process for preparing the nutritional supplement as defined herein, which comprises the step of reacting a sterol with an omega-3 fatty acid, or an ester thereof, in the presence of a base.

Base catalysts were found to be successful in the transesterification (or interesterification) process of the invention. Such a reaction is advantageous given the availability of esterified omega-3 fatty acid starting material, for example from fish oil. In addition, acidic catalysts were found to be ineffective in the transesterification of interest.

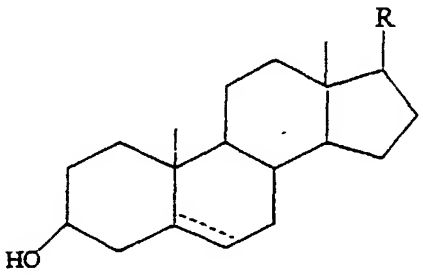
Sterols are not very soluble in lipid, which complicates their use in lipid-based foods. A mixture of a sterol and a free omega-3 fatty acid, which typically forms a paste at a molar ratio of 1:1, may be used. If a mixture is used, the omega-3 fatty acid can be a free acid or can be in ester form, preferably a succinimidyl, triglyceride, (C_3-C_{12})cycloalkyl or (C_1-C_8)alkyl ester, more preferably an ethyl ester. In the mixture, the molar ratio range of omega-3 fatty acid, or an ester thereof, to sterol should be about 0.5 to 8 , preferably 0.76 to 6.4, more preferably 1 to 2.

Preferably, the sterol and the omega-3 fatty acid are together in the form of an ester. The sterol esters of the

present invention are highly fat-soluble and represent a bifunctional species, since they lower both serum cholesterol and serum triglyceride levels in the bloodstream.

Detailed Description of the Preferred Embodiments

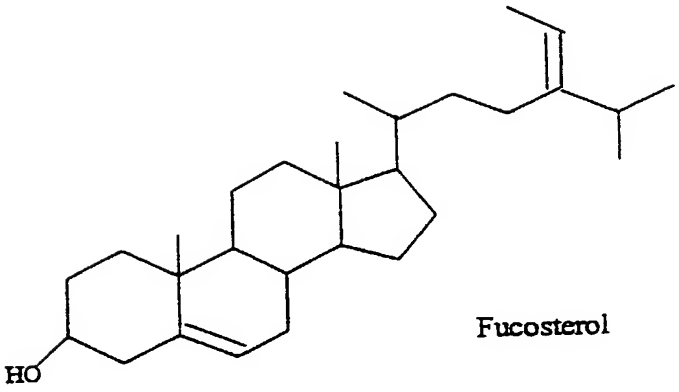
The sterols used to prepare the nutritional supplement of the present invention are preferably phytosterols, and preferably have a perhydrocyclopentanophenanthrene ring system as shown below in the compound of formula I:



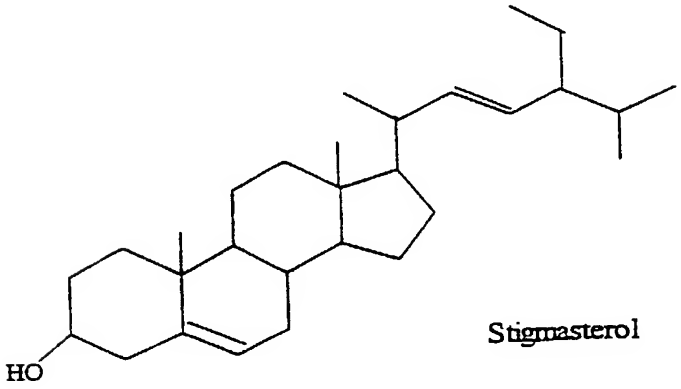
(I)

wherein the dashed line is a single or double bond and R is a (C_1-C_{10})alkyl, substituted (C_1-C_{10})alkyl, (C_2-C_{10})alkenyl or substituted (C_2-C_{10})alkenyl group.

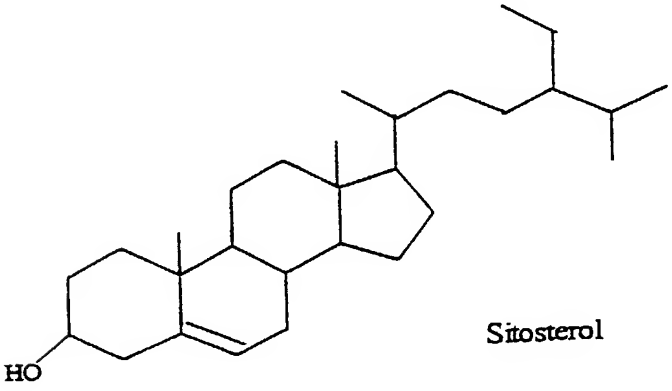
In the present application, the term "sterols" includes sterols in reduced form (stanols), preferably β-sitostanol or fucostanol (reduced fucosterol).



Fucosterol



Stigmasterol

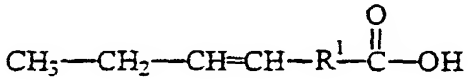


Sitosterol

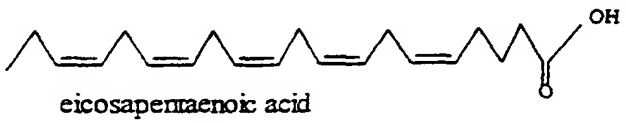
One or more sterols can be used to prepare the nutritional supplement. The term "phytosterols" includes sterols from terrestrial or marine plants, seaweed, microalgae, etc. Preferably, the sterol is stigmasterol, sitosterol, fucosterol, β-sitostanol or fucostanol.

Fucosterol is abundant in brown algae. Prior to esterification with the omega-3 fatty acid, fucosterol can be reduced to fucostanol. Preferably, the reduction is carried out using hydrogen gas in the presence of a suitable catalyst such as palladium on charcoal (Pd/C), but other reduction processes that ultimately yield a food-quality ester, after purification if necessary, may be used.

The nutritional supplement of the present invention comprises one or more omega-3 fatty acids, and is preferably an ester of an acid of the formula:



wherein R^1 is a (C_3-C_{40})alkenylene group comprising at least one double bond, more preferably 2 to 5 double bonds. More preferably, the omega-3 fatty acid is stearidonic acid 18:4ω3 (SA), eicosapentaenoic acid 20:5ω3 (EPA) or docosahexaenoic acid 22:6ω3 (DHA).



eicosapentaenoic acid

docosahexaenoic acid

Omega-3 fatty acids, such as EPA and DHA, are long-chain polyunsaturated fatty acids (LCPUFAs) that are abundant in oily fish such as menhaden, salmon, tuna, and sardine, as well as in certain plants and microbes, such as particular fungi and microalgae. The preferred source of omega-3 fatty acids for the present invention is fish oil, more preferably a highly refined fish oil concentrate having approximately 65% omega-3 fatty acid content which is predominantly EPA and DHA in the form of triglyceride esters. These triglycerides are preferably converted to lower alkyl esters, such as methyl, ethyl or propyl esters, by known methods and used in an esterification with a sterol to form esters, which can be further purified if necessary, for use as nutritional supplements.

The cardiovascular effects of dietary fish oils have long been recognized [18, 19]. Omega-3 fatty acids lower plasma triglyceride concentrations principally by inhibiting synthesis of triacylglycerol and VLDL by the liver [20]. In addition, omega-3 fatty acids are anti-thrombotic and are protective against cardiac arrhythmias [21]. The benefits of fish oil consumption are illustrated by the finding of the Diet and Reinfarction Trial (DART) which showed a reduction of 29% in the overall mortality in survivors of a first myocardial infarction who consumed fish rich in omega-3 fatty acids at least twice weekly [22]. Two recent studies demonstrate the

efficacy of omega-3 fatty acid supplementation. In a randomized, double-blind, placebo-controlled trial patients with coronary artery disease who ingested a 1.5g/day fish oil supplement (55% EPA and DHA) for two years had less progression and more regression of their disease based on coronary angiography compared to patients ingesting the placebo [23]. In the GISSI- Prevenzione trial, omega-3 fatty acid supplements in patients who had myocardial infarction reduced cardiovascular death by 30% [24]. Although omega-3 fatty acids are anti-atherogenic, they do not lower plasma cholesterol and in some incidences may slightly increase LDL-cholesterol [25]. Safety and toxicological studies spanning several years have shown that fish oils are safe to consume. Recently, fatty acids such as the omega-3 fatty acids from fish oil were granted GRAS (Generally Regarded As Safe) status in the United States, which permits their addition to foods low in long-chain polyunsaturated fatty acids. The typical North American diet contains about 0.15 grams omega-3 fatty acids whereas Inuit may ingest up to 10 grams of omega-3 fatty acids daily. A daily intake of 2 to 3 grams of omega-3 fatty acids has consistently been shown to lower plasma triglycerides [18]. Therefore, a suitable daily intake of omega-3 fatty acid in the present invention is about 0.1 to about 10 grams, preferably about 2 to about 3 grams, but clearly greater amounts can be tolerated, and may be beneficial.

Phytosterols are considered safe for human consumption. A typical daily intake in North America is about 100 to 300 milligrams. However, a dose of greater than 3 grams of the phytosterol esters are required to have significant impact on plasma cholesterol levels [13]. Such doses are safe with no known side effects. In the present invention, a

preferred daily intake of phytosterol is about 2 to about 3 grams.

Phytosterol esters prepared using fish oil as the source of omega-3 fatty acids contain a significant amount of EPA and DHA. Such esters can simultaneously reduce serum cholesterol and serum triglyceride levels. The triglyceride-lowering ability of the omega-3 fatty acid component of the ester is dependent on its entry into the circulatory system. A lipid esterase in the intestinal lumen may be responsible for release of the omega-3 fatty acid from the phytosterol, which would make both species available for uptake into the circulatory system. There is a non-specific lipid esterase, secreted into the intestinal lumen during digestion that is active against a variety of molecular species including cholesterol esters, monoglycerides, and esters of vitamin A [26].

At least one edible additive, such as listed below, can be included for consumption with the nutritional supplement of the invention and may have, for example, antioxidant, dispersant, antimicrobial, or solubilizing properties. A suitable antioxidant is, for example, vitamin C, vitamin E or rosemary extract. A suitable dispersant is, for example, lecithin, an alkyl polyglycoside, polysorbate 80 or sodium lauryl sulfate. A suitable antimicrobial is, for example, sodium sulfite or sodium benzoate. A suitable solubilizing agent is, for example, a vegetable oil such as sunflower oil, coconut oil, and the like, or mono-, di- or tri-glycerides.

Additives include vitamins such as vitamin A (retinol, retinyl palmitate or retinol acetate), vitamin B1 (thiamin, thiamin hydrochloride or thiamin mononitrate),

vitamin B2 (riboflavin), vitamin B3 (niacin, nicotinic acid or niacinamide), vitamin B5 (pantothenic acid, calcium pantothenate, d-panthenol or d-calcium pantothenate), vitamin B6 (pyridoxine, pyridoxal, pyridoxamine or pyridoxine hydrochloride), vitamin B12 (cobalamin or cyanocobalamin), folic acid, folate, folacin, vitamin H (biotin), vitamin C (ascorbic acid, sodium ascorbate, calcium ascorbate or ascorbyl palmitate), vitamin D (cholecalciferol, calciferol or ergocalciferol), vitamin E (d-alpha-tocopherol, d-beta-tocopherol, d-gamma-tocopherol, d-delta-tocopherol or d-alpha-tocopheryl acetate) and vitamin K (phylloquinone or phytonadione).

Other additives include minerals such as boron (sodium tetraborate decahydrate), calcium (calcium carbonate, calcium caseinate, calcium citrate, calcium gluconate, calcium lactate, calcium phosphate, dibasic calcium phosphate or tribasic calcium phosphate), chromium (GTF chromium from yeast, chromium acetate, chromium chloride, chromium trichloride and chromium picolinate) copper (copper gluconate or copper sulfate), fluorine (fluoride and calcium fluoride), iodine (potassium iodide), iron (ferrous fumarate, ferrous gluconate or ferrous sulfate), magnesium (magnesium carbonate, magnesium gluconate, magnesium hydroxide or magnesium oxide), manganese (manganese gluconate and manganese sulfate), molybdenum (sodium molybdate), phosphorus (dibasic calcium phosphate, sodium phosphate), potassium (potassium aspartate, potassium citrate, potassium chloride or potassium gluconate), selenium (sodium selenite or selenium from yeast), silicon (sodium metasilicate), sodium (sodium chloride), strontium, vanadium (vanadium sulfate) and zinc (zinc acetate, zinc citrate, zinc gluconate or zinc sulfate).

Other additives include amino acids, peptides, and related molecules such as alanine, arginine, asparagine, aspartic acid, carnitine, citrulline, cysteine, cystine, dimethylglycine, gamma-aminobutyric acid, glutamic acid, glutamine, glutathione, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine and valine.

Other additives include animal extracts such as cod liver oil, marine lipids, shark cartilage, oyster shell, bee pollen and d-glucosamine sulfate.

Other additives include unsaturated free fatty acids such as γ-linoleic, arachidonic and α-linolenic acid, which may be in an ester (e.g. ethyl ester or triglyceride) form.

Other additives include herbs and plant extracts such as kelp, pectin, Spirulina, fiber, lecithin, wheat germ oil, safflower seed oil, flax seed, evening primrose, borage oil, blackcurrant, pumpkin seed oil, grape extract, grape seed extract, bark extract, pine bark extract, French maritime pine bark extract, muira puama extract, fennel seed extract, dong quai extract, chaste tree berry extract, alfalfa, saw palmetto berry extract, green tea extracts, angelica, catnip, cayenne, comfrey, garlic, ginger, ginseng, goldenseal, juniper berries, licorice, olive oil, parsley, peppermint, rosemary extract, valerian, white willow, yellow dock and yerba mate.

Other additives include enzymes such as amylase, protease, lipase and papain as well as miscellaneous substances such as menaquinone, choline (choline bitartrate), inositol, carotenoids (beta-carotene, alpha-carotene, zeaxanthin, cryptoxanthin or lutein), para-aminobenzoic acid, betaine HCl,

free omega-3 fatty acids and their esters, thiotic acid (alpha-lipoic acid), 1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid, alkyl polyglycosides, polysorbate 80, sodium lauryl sulfate, flavanoids, flavanones, flavones, flavonols, isoflavones, proanthocyanidins, oligomeric proanthocyanidins, vitamin A aldehyde, a mixture of the components of vitamin A_2, the D Vitamins (D_1, D_2, D_3 and D_4) which can be treated as a mixture, ascorbyl palmitate and vitamin K_2.

The nutritional supplement of the invention is typically a viscous oil and can be added to a foodstuff composition during processing of the foodstuff. Such a foodstuff composition is often referred to as a functional food, and can be any food that will tolerate the physicochemical properties of the nutritional supplement, for example, margarine, cooking oil, shortening or mayonnaise. It can also be packaged for consumption in softgel, capsule, tablet or liquid form. It can be supplied in edible polysaccharide gums, for example carrageenan, locust bean gum, guar, tragacanth, cellulose and carboxymethylcellulose.

The nutritional supplement can also be microencapsulated. Microencapsulation can be carried out, for example, using a gelatin such as bovine gelatin in a co-extrusion process, prior to processing into a foodstuff composition, for example baked goods, candy, margarines and spreads, ice cream, yogurts, frozen desserts, cake mixes and pudding mixes. The packaging of the nutritional supplement should preferably provide physical protection from such effects as pH, particularly basic conditions, oxidation and degradation by light. This latter effect can be minimized for example by changing the mesh size of the microencapsulation or inclusion

of a suitable dye. The nutritional supplement can also be stored in a light-opaque container to minimize photodegradation.

The example below describes synthesis of an ester of the invention. The ester linkage can be formed according to known methods, such as by esterification of free fatty acids by sterols or stanols under acid catalysis (US Patent No. 5,892,068: Higgins III, issued April 6, 1999). Preferably, however, a base is used as a catalyst to promote transesterification. More preferably, the base is a metal (C_1-C_{10})alkoxide, even more preferably sodium methoxide or ethoxide. Conveniently, the reactants are heated to a temperature of about 100°C to about 200°C with stirring, preferably under reduced pressure, for about 30 minutes to about 4 hours. The base is then added and the mixture conveniently stirred at a temperature of about 100°C to about 200°C under reduced pressure for about 30 minutes to about 36 hours. Alternatively, the starting ester is heated to a temperature of about 100°C to about 200°C with stirring, preferably under reduced pressure, for about 30 minutes to about 4 hours. The base dispersed in the phytosterol is then added and the mixture conveniently stirred at a temperature of about 100°C to about 200°C under reduced pressure for about 30 minutes to about 36 hours. The ester that is formed can be further purified if necessary for use as a nutritional supplement.

The further purification is preferably carried out by precipitation and extraction, preferably sequentially, using two immiscible solvents. Unreacted sterol is precipitated by addition of a suitable non-polar solvent and filtered off. A suitable non-polar solvent can be an aliphatic liquid such as a

liquid alkane, preferably pentane, hexane, heptane, octane, isooctane or dodesane, more preferably hexane. Corresponding fluoroalkanes can also be used. The non-polar solvent can also be an aromatic solvent such as benzene or toluene, or an other solvent of similar polarity such as carbon tetrachloride or methyl-tert-butyl ether.

The filtrate is then extracted by a suitable extraction solvent to remove unreacted omega-3 fatty acid-containing material. The extraction solvent is preferably a polar solvent such as methanol, ethanol or ethylene glycol dimethyl ether (monoglyme), more preferably methanol. Certain dipolar aprotic solvents, such as N,N-dimethyl formamide (DMF) or dimethylsulfoxide (DMSO), can also be used.

Example 1

Synthesis of Stigmasterol/Omega-3 Fatty Acid Esters.

(A) A mixture of dry stigmasterol (3 g, 7.27 mmol) and a highly concentrated mixture of EPA and DHA omega-3 fatty acids in ethyl ester form (EPAX™ 5500, ProNova; 4.3 g, 12.6 mmol) were heated while being stirred magnetically at 140 to 145°C for 2 hours under vacuum (5 mm). Subsequently the vacuum was disconnected and powdered sodium methoxide (40 mg, 0.75 mmol) was added quickly in one portion. The vacuum was connected immediately and the mixture was stirred at 140 to 145°C for an additional 4 hours. Hexane (25 mL) was added to precipitate the residual stigmasterol and the mixture was centrifuged for 5 minutes at 15,000 g (0°C), the supernatant was removed and the pellet was washed again with 5 mL of hexane. The remaining precipitate was centrifuged off and the supernatants combined. The organic phase was washed with water

(5 mL), dried over sodium sulfate and the solvent removed under reduced pressure. TLC (hexane/diethylether/acetic acid (90:10: 1), R_f 0.71. The yield was 5.9 g (85%). The ester product was a viscous oil.

When the experiment was repeated using freshly made sodium ethoxide, almost the same level of conversion was obtained as with sodium methoxide. However, this was not seen with commercially available sodium ethoxide, which performed more poorly than sodium methoxide.

Synthesis of Stigmasterol/Omega-3 Fatty Acid Esters

(B) A highly concentrated mixture of EPA and DHA omega-3 fatty acids in ethyl ester form (EPAX™ 5500 EE, BioNova; 221 g, 649 mmol) was heated while being stirred magnetically at 140 to 145°C for 2 hours under vacuum (5 mm). A well dispersed mixture of dry stigmasterol (268g, 649 mmol) and sodium methoxide (40 mg, 0.75 mmol) was added portionwise within 1 hour and the mixture was stirred at 170 to 175°C for an additional 21 hours. The reaction mixture was liberated from unreacted material either by column chromatography (2% diethylether in hexane on silicagel) or by a sequential extraction using two immissible solvents. The unreacted stigmasterol was precipitated upon addition of hexane and the solution was then filtered. The filtrate was extracted with methanol to remove unreacted starting oil material. TLC (hexane/diethylether/acetic acid (90:10: 1) gave an R_f equal to 0.71. The yield was 434 g (70 %). The ester product was a viscous oil.

When the experiment was repeated using freshly made sodium ethoxide, almost the same level of conversion was

obtained as with sodium methoxide. However, this was not seen with commercially available sodium ethoxide, which performed more poorly than sodium methoxide.

The procedure works also from a concentrated mixture of EPA and DHA omega-3 fatty acids in triglyceride form (EPAX™ 5500 TG, BioNova) with a similar yield of final product.

Example 2

The effect of a phytosterol-fish oil ester-containing diet on plasma lipid levels in guinea pigs.

Guinea pigs were chosen for this project, as their blood lipid profiles and responses to dietary manipulation more closely resemble those of humans than do more commonly used laboratory rodents. Two groups of eight guinea pigs each were fed a standard, non-purified guinea pig chow (Prolab guinea pig 5P18, PMI Nutrition International, Inc., Brentwood, MO). Baseline values for blood lipids were determined and then the animals were placed on a control diet (Group 1) or a phytosterol-fish oil ester-containing diet (Group 2).

Phytosterol-fish oil esters were prepared as described in Example 1 and mixed 5:1 with corn oil. This was incorporated into crushed chow to give a concentration of phytosterol-fish oil esters of 2.5% (w/w). Control diet was prepared using an equivalent amount of corn oil. Both control and test diets were supplemented with 0.08% cholesterol. The chow was re-pelleted using a Hobart extruder. Food was stored in sealed plastic bags with nitrogen purging at -20°C in the dark. Fresh food was prepared each week.

Blood samples were collected from each animal after 2 and 4 weeks for determination of plasma lipids (total cholesterol, HDL-cholesterol, non-HDL-cholesterol, and triacylglycerols).

Guinea pigs fed phytosterol-fish oil esters (2.5% g/100 gram diet) had significantly lower levels of plasma total cholesterol and triacylglycerol compared to control fed animals after 4 weeks of feeding (Table 1). At this time, plasma cholesterol and triacylglycerols were 36% and 29% lower in the treatment group. A statistically significant effect of phytosterol-fish oil esters on cholesterol was also evident after 2 weeks where the reduction was 30% compared to the control value. The changes in cholesterol level could be completely explained by changes in the amount of non-high density lipoprotein (HDL)-cholesterol (Table 2). Non-HDL cholesterol was 30% and 38% lower in the phytosterol-fish oil ester-fed group at 2 and 4 weeks, respectively, whereas there were no differences in HDL-cholesterol.

These results illustrate the ability of dietary phytosterol-fish oil esters to reduce the levels of plasma cholesterol and triacylglycerol. It is also shown that phytosterol-fish oil esters lower non-HDL cholesterol (*"bad cholesterol"*) but do not affect the level of HDL (*"good cholesterol"*).

Table 1.

The effect of a phytosterol/fish oil esters containing diet on plasma total cholesterol and triacylglycerol levels in guinea pigs

		Total Cholesterol	Triacylglycerol
Group 1	Week 2	1.72 ± 0.38	0.92 ± 0.26
	Week 4	2.05 ± 0.20	0.87 ± 0.16
Group 2	Week 2	1.22 ± 0.10 *	0.77 ± 0.22
	Week 4	1.32 ± 0.20 *	0.62 ± 0.13 *

Results are mean ± S.D. of 8 guinea pigs per group. The baseline values for plasma total cholesterol and triacylglycerol were 1.28 ± 0.12 (mM) and 0.65 ± 0.11 (mM) respectively.

*Significantly lower than the corresponding value for Group 1 ($p < 0.05$; Bonferroni's Multiple Comparison Test).

Table 2.

The effect of a phytosterol/fish oil esters containing diet on lipoprotein metabolism in guinea pigs

		HDL Cholesterol	non-HDL Cholesterol
Group 1	Week 2	0.14 ± 0.03	1.58 ± 0.4
	Week 4	0.16 ± 0.06	1.90 ± 0.2
Group 2	Week 2	0.11 ± 0.04	1.11 ± 0.14 *
	Week 4	0.16 ± 0.03	1.17 ± 0.23 *

Results are mean ± S.D. of 8 guinea pigs per group. The baseline values for HDL cholesterol and non-HDL cholesterol were 0.16 ± 0.07 (mM) and 1.14 ± 0.16 (mM) respectively.

*Significantly lower than the corresponding value for Group 1 ($p < 0.05$; Bonferroni's Multiple Comparison Test).

Example 3.

The effect of a phytosterol-fish oil ester-containing diet on plasma lipid levels in an obese rat model

The efficacy of a phytosterol-fish oil ester-containing diet to lower plasma triacylglycerol and cholesterol was studied in the JCR:La-cp (corpulent) rat, a genetic model of obesity (O'Brien and Russell, 1997). Animals of this strain, if homozygous for the autosomal recessive cp gene (cp/cp), are obese, insulin resistant, hyperinsulinemic, and highly hypertriglyceridemic. In addition the obese animals exhibit poor vascular responsiveness and develop ischemic lesions of the myocardium with age. Rats that are homozygous normal or heterozygous (-/?), are lean and metabolically normal. The effect of phytosterol-fish oil ester feeding was determined using obese (cp/cp) rats at 8 weeks of age, when the rats are clearly obese and fully insulin resistant. Lean litermates (+/?) of the obese animals were included in the study as benchmark for comparison. Obese animals were fed one of four diets: a control diet containing no added oil (Group 1); a control diet containing 2.6 g/kg canola (Group 2); or diets containing 0.5 or 2.6 g/kg phytosterol-fish oil ester (Group 3 and Group 4, respectively). The lean animals (Group 5) received the control without canola. The various test diets were fed for four weeks.

Preparation of the diets using standard rat chow (Rodent Diet 5001, PMI Nutrition International, St Louis, Mo) was essentially the same as described in Example 2. Phytosterol-fish oil ester was mixed with canola oil (5:1) and the oil mixture was added to the powered diet at a concentration of 0.5 g/kg or 2.6 g phytosterol ester/kg diet,

which was then pelleted. Control diets contained no added oil or 2.6 g/kg canola oil. Food was stored in sealed plastic bags with nitrogen purging and maintained at 4°C. Fresh food was prepared each week.

Blood samples were collected from each animal at the start and after 4 weeks for determination of plasma lipids (total cholesterol, cholesterol esters, phospholipids, and triacylglycerols).

Obese JCR-La rats exhibit marked hypertriglyceridemia and elevated plasma cholesterol levels compared to their lean littermates (Group 1 or 2 versus Group 5; Table 3). There was a concentration-dependent effect of dietary phytosterol-fish oil esters on plasma lipid concentrations. The lower dose of 0.5 g phytosterol-fish oil ester/kg food had no impact on lipid parameters in animals fed for 4 weeks (Group 3 versus Group 2 at 12 weeks; Table 3). However 2.6 g phytosterol-fish oil ester /kg food reduced triacylglyerol level from control levels by 51% (1.26 mM versus 2.59 mM in the control). Although this is a marked reduction, the animals are still strongly hypertriglyceridemic (Group 4 versus Group 5). There was also a modest reduction of cholesterol levels in animals fed the high dose of phytosterol-fish oil ester (13% reduction in total cholesterol; 17% reduction in cholesterol esters). There was a tendency for phospholipid values to be reduced in phytosterol-fish oil ester-fed animals but this did not reach statistical significance.

The results show that phytosterol-fish oil esters decrease plasma triacylglyerol and cholesterol in obese JCR-La rats and that this occurs in a dose-dependent manner. The reduction in triacylglycerol and cholesterol esters is

consistent with a substantial reduction in very low density lipoprotein (VLDL) particles through a decreased rate of VLDL production by the liver. These improvements in lipid profile might also be expected to have a beneficial effect on the insulin-resistant state of these animals.

Table 1. Whole serum lipid concentrations in high dose ON-1-treated male JCR-LA-cp rats

	Free Cholesterol	Cholesteryl esters	Total cholesterol	Phospholipids	Triacylglycerols
Initial values at 8 weeks of age:					
Group 1 (no oil control)	0.73 ± 0.11	1.19 ± 0.39	2.63 ± 0.49	2.19 ± 0.36	2.06 ± 1.19
Group 2 (oil control)	0.68 ± 0.10	1.89 ± 0.31	2.58 ± 0.40	2.01 ± 0.20	1.37 ± 0.63
Group 3 (0.5 mg/kg dose)	0.75 ± 0.12	2.01 ± 0.19	2.76 ± 0.30	2.35 ± 0.33	2.17 ± 1.11
Group 4 (2.6 mg/kg dose)	0.74 ± 0.09	1.94 ± 0.24	2.67 ± 0.33	2.28 ± 0.27	2.64 ± 0.84
Group 5 (lean control)	0.48 ± 0.06	1.31 ± 0.09	1.79 ± 0.12	1.01 ± 0.13	0.25 ± 0.16
Final values at 12 weeks of age:					
Group 1 (no oil control)	0.67 ± 0.06	1.58 ± 0.24	2.25 ± 0.29	1.92 ± 0.27	2.58 ± 0.93
Group 2 (oil control)	0.60 ± 0.09	1.61 ± 0.16	2.21 ± 0.23	1.87 ± 0.22	2.59 ± 0.58
Group 3 (0.5 mg/kg dose)	0.62 ± 0.14	1.55 ± 0.26	2.17 ± 0.37	1.90 ± 0.26	2.51 ± 0.71
Group 4 (2.6 mg/kg dose)	0.58 ± 0.06	1.34 ± 0.11**	1.92 ± 0.15*	1.66 ± 0.19	1.26 ± 0.72**
Group 5 (lean control)	0.34 ± 0.03	0.90 ± 0.04	1.24 ± 0.06	0.71 ± 0.04	0.17 ± 0.04

Values are mmol/l; mean ± S.D., 8 rats in each group. ** Significantly lower compared to group 2(P<0.05).

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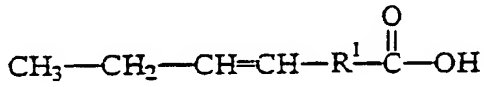
CLAIMS:

1. A nutritional supplement comprising an ester formed between a sterol and an omega-3 fatty acid for lowering cholesterol and triglyceride levels in the bloodstream of a subject.

2. The nutritional supplement according to claim 1, wherein the sterol is a phytosterol.

3. The nutritional supplement according to claim 1 or 2, wherein the omega-3 fatty acid has the formula:



wherein R^1 is a (C_3-C_{40})alkenylene group comprising at least one double bond.

4. The nutritional supplement according to claim 3, wherein R^1 has from 2 to 5 double bonds.

5. The nutritional supplement according to claim 4, wherein the omega-3 fatty acid is eicosapentaenoic acid 20:5ω3 (EPA).

6. The nutritional supplement according to claim 4, wherein the omega-3 fatty acid is docosahexaenoic acid 22:6ω3 (DHA).

7. The nutritional supplement according to any one of claims 1 to 6, wherein the sterol is a phytosterol.

8. The nutritional supplement according to any one of claims 1 to 7, wherein the sterol is stigmasterol.

9. The nutritional supplement according to any one of claims 1 to 7, wherein the sterol is sitosterol.

10. The nutritional supplement according to any one of claims 1 to 7, wherein the sterol is fucosterol.

11. The nutritional supplement according to any one of claims 1 to 7, wherein the sterol is fucostanol.

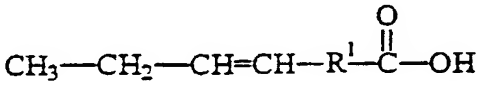
12. The nutritional supplement according to any one of claims 1 to 7, wherein the sterol is β-sitostanol.

13. The nutritional supplement according to any one of claims 1 to 12, further comprising an edible additive.

14. A method of lowering cholesterol and triglyceride levels in the bloodstream of a subject, the method including the step of administering to a subject an effective amount of a nutritional supplement comprising an ester formed between a sterol and an omega-3 fatty acid.

15. The method according to claim 14, wherein the omega-3 fatty acid is derived from fish oil.

16. The method according to claim 14 or 15, wherein the omega-3 fatty acid has the formula:



wherein R^1 is a (C_3-C_{40})alkenylene group comprising at least one double bond.

17. The method according to claim 16, wherein R^1 has from 2 to 5 double bonds.

18. The method according to claim 17, wherein the omega-3 fatty acid is eicosapentaenoic acid 20:5ω3 (EPA).

19. The method according to claim 17, wherein the omega-3 fatty acid is docosahexaenoic acid 22:6ω3 (DHA).

20. The method according to any one of claims 14 to 19, wherein the sterol is a phytosterol.

21. The method according to any one of claims 14 to 20, wherein the sterol is stigmasterol.

22. The method according to any one of claims 14 to 20, wherein the sterol is sitosterol.

23. The method according to any one of claims 14 to 20, wherein the sterol is fucosterol.

24. The method according to any one of claims 14 to 20, wherein the sterol is fucostanol.

25. The method according to any one of claims 14 to 20, wherein the sterol is β-sitostanol.

26. Use of a nutritional supplement comprising an ester formed between a sterol and an omega-3 fatty acid, as defined in any one of claims 1 to 13, for lowering cholesterol and triglyceride levels in the bloodstream of a subject.

27. A foodstuff having a nutritional value enhanced by incorporation of the nutritional supplement according to any one of claims 1 to 13.

28. Use of the nutritional supplement according to any one of claims 1 to 13 in the manufacture of a foodstuff.

29. A process for preparing the nutritional supplement as defined in any one of claims 1 to 13, which comprises the step of reacting a sterol with an omega-3 fatty acid, or an ester thereof, in the presence of a base.

30. A process according to claim 29 wherein the base is a metal (C_1-C_{10}) alkoxide.

31. A process according to claim 30, wherein the metal (C_1-C_{10}) is sodium methoxide.

32. A process according to claim 29, 30 or 31, which further comprises the step of precipitating unreacted sterol with a suitable non-polar solvent, and filtering off the precipitated unreacted sterol to leave a filtrate.

33. A process according to claim 32, wherein the non-polar solvent is hexane.

34. A process according to claim 32 or 33, which further comprises the step of extracting the filtrate with a suitable immiscible solvent to remove unreacted omega-3 fatty acid, or an ester thereof, from the filtrate.

35. A process according to claim 34, wherein the immiscible solvent is methanol.

36. A process according to any one of claims 29 to 35, wherein the ester of the omega-3 fatty acid is a triglyceride ester.

37. A process according to any one of claims 29 to 35, wherein the ester of the omega-3 fatty acid is an ethyl ester.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/15552 A1

(51) International Patent Classification[7]: A23L 1/30, A61K 31/575, C11C 3/00, C07J 9/00

(21) International Application Number: PCT/CA00/01011

(22) International Filing Date: 30 August 2000 (30.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/385,834 30 August 1999 (30.08.1999) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/15552 A1

(54) Title: A NUTRITIONAL SUPPLEMENT FOR LOWERING SERUM TRIGLYCERIDE AND CHOLESTEROL LEVELS

(57) Abstract: Triglycerides and cholesterol in the bloodstream are important factors in the development of cardiovascular disease. The present invention discloses a nutritional supplement comprising a sterol and an omega-3 fatty acid, or an ester thereof, for lowering cholesterol and triglyceride levels in the bloodstream of a subject. Preferably, the sterol and omega-3 fatty acid are together in the form of an ester.

78162-37 / ala

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A NUTRITIONAL SUPPLEMENT FOR LOWERING SERUM TRIGLYCERIDE AND CHOLESTEROL LEVELS

the specification of which

☐ is attached hereto.

☐ was filed on _____

as U.S. Application Serial No. _____

☒ was filed on August 30, 2000

as PCT International Application No. PCT/CA00/01011

and (if applicable) was amended on November 1, 2001 and February 27, 2002

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §§1.56(a) and (b), which state:

"(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

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A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability."

I hereby claim foreign priority benefits under 35 United States Code, §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing of this application:

PRIOR FOREIGN APPLICATION(S)

Number	Country	Filing Date *(Day/Month/Year)*	Date First Laid-open or Published	Date Patented or Granted	Priority Claimed?

I hereby claim the benefit under 35 United States Code, §119(e) of any United States provisional application(s) listed below:

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

PRIOR U.S. OR PCT APPLICATION(S)

Application No.	Filing Date *(day/month/year)*	Status *(pending, abandoned, granted)*
09/385,834	30 August 1999	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following patent agents with full power of substitution, association and revocation to prosecute this application and/or international application and to transact all business in the Patent and Trademark Office connected therewith:

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